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cell contains a special genetic substance or structure, differentiated to perform genetic functions ... " (Taylor, 1949).

CORRELATING CYTOLOGICAL AND GENETIC INVESTIGATIONS

Other speakers at this symposium will have discussed in more detail the present status of bacterial cytology and its bearings on bacterial genetics. A number of workers have presented convincing evidence for the presence of nuclei in bacterial cells, but their identification as nuclei has hitherto been based only on incomplete morphological and cytochemical evidence, in the shaence of any more direct opportunity to locate the genes within them. A most attractive objective would be a documentation of the nuclear events associated with genetic recombination in E. coli K-12, or any other suitable organism, but this is on the horizon, not at hand.

Meanwhile, many investigations of mutagenesis have been predicated on probably fallacious models of bacterial cells as constructively isolated genes, despite the contrary cytological evidence for the multi-nucleate condition of most rod-shaped bacteria. Many of the characters used in bacterial mutation research are recessive (e.g., resistance to phage or streptomycin) so that mutations induced in multinucleate cells could not begin to exert their phenotypic effect until nuclear separation has occurred. In this respect a comparison of vegetative cells with presumably uninucleate endospores might be fruitful.

The establishment of nondisjunctional or "diploid" cultures opened the question of a cytological comparison of 2n and n for the purposes of a bacterial cytogenetics. For some time, preparations like that illustrated in Figure 5, have encouraged this hope. Diploids often show cells of greater uniform length than haploids, and with chromatinic structures of greater apparent

complexity. Very often, there appeared to be two larger, more dispersed "nuclei" per cell, in contrast to two pairs of more condensed nuclei that often characterize comparable haploid cultures. The structure of the "nuclei" is obscure, for we had been unable to analyse the connections of the granules to determine whether they form a single connected group or several groups. So far, our material, interpretations, or techniques (HC!-Giemsa) have not sufficed to demonstrate clear mitotic figures, but there are many unmistakable examples of symmetrically placed groups of chromatin both in haploid and diploid cells. The preparations so far studied do not admit of any clear interpretation in terms of doubled chromosomes, and it is not yet excluded that the differences reside principally in a better expansion and resolution of nuclear structure in the diploid cells. In occasional preparations haploid cultures have shown nearly the same order of complexity in chromatinic structure as diploid (Figure 6), but to date one of us has consistently been able correctly to classify stained smears prepared by another, ostensibly by virtue of the nuclear cytology. On two occasions, a cytological determination correctly anticipated a later genetic definition of the status of a culture (one was a secondary Lac + homozygote: one a haploid culture carrying an unstable gene which simulated the variegation of heterozygosity). The further cytological analysis may well rest upon technical and conceptual advances of the kind discussed elsewhere in this symposium.

Stempen (1950) and others have reported that nuclei can be visualized in living bacteria by phase contrast microscopy. This technique has remarkable advantages for observing cells as a whole, but only faint suggestions of the nuclei are apparent in preparations of E. coli K-12 There is considerable fluctuation in the definition of the presumed chromatin (which appears light

PLATEI

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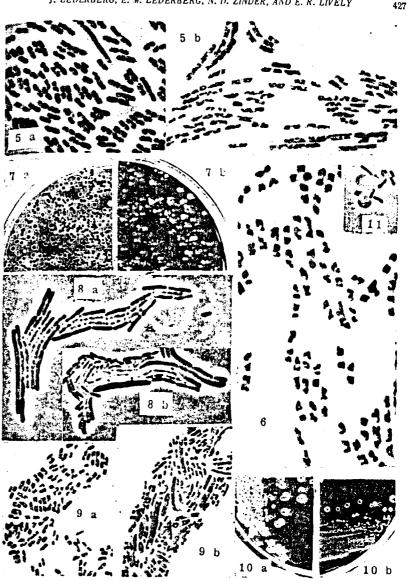


FIG. 5. Haploid parent, W-67 (a), and diploid offspring, H-226 (b). Giemsa stain following osmic fixation and hydrolysis with HCL

FIG. 6. Haploid cells, K-12. Giemsa-osmic-HCl.

FIG. 7. Genetic effects of ultra-violet light on a diploid culture, H-226. a. Control plating showing prepon derance of balanced lactose-positive colonies (Lac1-/Lac4-; see Figure 4C) on EMB lactose agar. b. Comparable plating of an aliquot exposed to ultra-violet light.

FIG. 8. Phase contrast photomicrographs of microcolonies, a. Control plating of strain K-12. b. From diploid

cells, If-267, exposed to ultra-violet light. FIG. 9. Cytological effects of ultra-violet light on a diploid culture, H-267. a. Microcolony from control sus-

pension. b. Microcolony from treated suspension. Giemsa-osmic-HCL FIG. 10. Mutability differences between Laci- alleles. a. Mutable, Y-87. b. Stable, W-112. Both on EMB

lactose agar, 48-hour plates. FIG. 11. "Large Bodies" from Salmonella filtrates, exposed to antiserum. The bacteria were artificially added to provide a size standard.